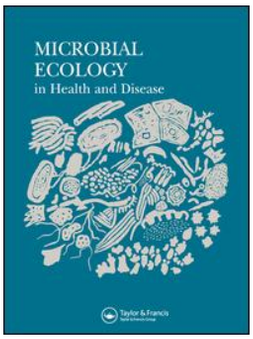

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Short Communication

Influence of Antimicrobial Drugs on Segmented Filamentous Bacteria in the Ileum of Mice

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The effects of various types of 3 d antibiotic treatment of mice on the presence of segmented filamentous bacteria (SFBs) in the ileum were measured in order to further characterise these microorganisms. To assess any specific effects of the antimicrobial drugs on SFBs, relative caecal weight, relative number of fusiform-shaped bacteria in the caecum and the number of faecal Enterobacteriaceae were also determined. All drugs tested, i.e. amoxycillin, doxycyclin, gentamicin, vancomycin, ciprofloxacin, trimethoprim, metronidazole, clindamycin, streptomycin and cefotaxim, reduced the presence of SFBs in the ileum, although to different degrees. None of the drugs affected body weight of the mice. There was no correlation of the drug effects on SFBs and those on either relative caecal weight, percentage of caecal fusiforms or faecal Enterobacteriaceae. Thus, the effects of the antimicrobial drugs on SFBs can be considered rather specific. The sensitivity pattern of SFBs suggests that they are facultatively anaerobic bacteria with relatively high sensitivity to antimicrobial drugs.

KEY WORDS—Segmented filamentous bacteria; Intestinal bacteria; Small intestine; Mouse; Antibiotics.

INTRODUCTION

Because they cannot be cultured *in vitro*, segmented filamentous bacteria (SFBs) inhabiting the distal small intestine of mice are poorly characterised.[1,3,11] These bacteria have also been demonstrated in animal species other than mice.[5,6,9] SFBs might influence the host's resistance to certain enteropathogenic bacteria.[5,9,19,24,27] Habitat and morphology of SFBs have been studied,[1,3,4] but metabolic characteristics are unknown.

SFBs in mice and rats are resistant to some antimicrobial drugs but sensitive to others.[4,8,15,16,18,20] The influence of antibiotics on SFBs has not been investigated systematically. Therefore, we determined a sensitivity pattern of SFBs towards ten different antibiotics. It was anticipated that the information thus obtained would contribute to the functional characterisation of SFBs. In order to assess the specificity of the response of SFBs to treatment of mice with antibiotics, we also measured three general parameters of the intestinal microbial ecology:[18,20] relative caecal weight (RCW), percentage of fusiform-shaped bacteria in the caecum and number of faecal Enterobacteriaceae.

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MATERIALS AND METHODS

Antimicrobial drugs

We selected 10 antibiotics, representing nine categories. The following were used. Aminopenicillins: Na-amoxycillin (Clamoxyl; Beecham Farma BV, Amstelveen, The Netherlands); tetracyclins: doxycyclin hydrochloride (Vibramycin I.V.; Pfizer BV, Rotterdam, The Netherlands); aminoglycosides: gentamicin sulphate (Garamycin; Essex Laboratories BV, Heist-op-den-Berg, Belgium) and streptomycin sulphate (Streptomycin sulphate;

Table 1. Dosage of antimicrobial drugs administered to mice

Antimicrobial drug	Mg/ml drinking water or mg/mouse/day*		
	Expt 1	Expt 2	Expt 3
Amoxycillin†	3·0 (18·40)	0·3 (2·50)	0·03 (0·29)
Doxycyclin†	0·22 (2·20)	0·022 (0·16)	0·002 (0·02)
Gentamicin†	0·3 (2·50)	0·03 (0·25)	0·003 (0·03)
Vancomycin†	0·75 (4·20)	0·075 (0·25)	0·0075 (0·06)
Ciprofloxacin†	0·75 (5·40)	0·075 (0·62)	0·0075 (0·08)
Trimethoprim†	0·38 (3·80)	0·038 (0·34)	0·0038 (0·04)
Metronidazole‡	1·5 (4·10)	0·15 (0·32)	0·015 (0·10)
Clindamycin†	0·08 (0·80)	0·008 (0·04)	0·0008 (0·009)
Streptomycin†	5·0 (28·00)	0·5 (4·40)	NS
Cefotaxim†§	— (10·00)	— (1·00)	— (0·10)
Control‖	—	—	—
Control with syrup¶	NS	—	—

*Dose, expressed as mg/mouse/day, is given in parentheses.
†Drinking water also contained 25 per cent (v:v) syrup.
‡Drinking water also contained 40 per cent (v:v) syrup.
§Cefotaxim was administered intraperitoneally.
‖Drinking water without additives.
¶Drinking water with 25 per cent (v:v) syrup.
NS = not studied.

Pharmachemie BV, Haarlem, The Netherlands); polypeptide antibiotics: vancomycin hydrochloride (Vancocin; Eli Lilly Int. Corp., Indianapolis, IN, USA); quinolones: ciprofloxacin lactate (Ciproxin; Bayer, Leverkusen, Germany); diaminopyrimidines: trimethoprim (Trimethoprim; Pharmachemie BV, Haarlem, The Netherlands); nitroimidazol derivatives: metronidazole (Metronidazol; NPBI, Emmer-Compascuum, The Netherlands); lincomycin group: clindamycin phosphate (Dalacin C; Upjohn, Ede, The Netherlands) and cephalosporins: Na-cefotaxim (Claforan; Roussel BV, Hoevelaken, The Netherlands).

Table 1 shows the dosage given to mice in three separate experiments. The doses in experiment 1 were based on human therapeutic dosage, except for streptomycin which was dosed according to other investigators.[7,8,18,25,26] Cefotaxim was injected intraperitoneally. All other antibiotics were dissolved in drinking water in amounts equivalent to about one human daily dose per litre. The concentrations in experiments 2 and 3 were 10 per cent and 1 per cent of those in experiment 1 (Table 1).

To improve taste, *sirupus rubi idaei* (Brocacef BV, Maarssen, The Netherlands) was added to the drinking water to concentrations of either 25 or 40 per cent (v:v). The former concentration corresponded to ca. 160 g saccharose/l water. To take into account any effects of the syrup, one control group received drinking water with 25 per cent syrup (Table 1). The antibiotics were administered over 3 d.

Animals and housing conditions

The number of mice used is indicated in Table 2. Female Cpb:SE (Swiss) mice, 7 wk old and with an SPF flora,[22] were used. Body weight was on average 20 g. The mice were housed individually in wire-topped type II macrolon cages (RUCO Metaalindustrie Nederland BV, Valkenswaard, The Netherlands). All mice were supplied *ad libitum* with demineralised sterilised water and a home-made[17] pelleted diet. Room temperature was 20–22°C, relative humidity 60–70 per cent and light was on from 06.00 to 18.00 h.

Measurements

Individual body weights were measured daily. Daily water consumption per animal was measured to determine antibiotic intake. After 3 d, all mice

Table 2. Influence of antimicrobial drugs on SFBs in mice

Antimicrobial drug†	Expt 1		Expt 2		Expt 3	
	SFB score‡	Incidence§	SFB score‡	Incidence§	SFB score‡	Incidence§
Amoxycillin	ND	0/2	ND	0/2	ND*·**	0/8
Doxycyclin	ND	0/2	ND	0/2	ND*·**	0/8
Gentamicin	ND[a]	0/2	ND[a]	0/2	35±22[b]	13/14
Vancomycin	ND	0/2	ND	0/2	0·3±0·5*·**	4/14
Ciprofloxacin	ND	0/2	ND	0/2	ND*·**	0/8
Trimethoprim	ND[a]	0/2	37±27[b]	8/8	12±21[a,*]	6/8
Metronidazole	ND[a]	0/2	10±14[b]	7/8	33±23[b]	7/8
Clindamycin	ND	0/2	ND	0/2	ND*·**	0/8
Streptomycin	ND*	0/6	ND*	0/6	NS	
Cefotaxim	ND	0/2	ND	0/2	ND*·**	0/8
Control	18±4	2/2	42±32	6/8	44±20	8/8
Control with syrup	NS		15±21	1/2	17±18*	7/8

†For dosage of antimicrobial drugs, see Table 1.
‡Percentage SFB-positive fields in Gram-stained mucosal smears of the ileum; means ± SD.
§Number of SFB-positive mice/total number of mice.
ND = not detectable, NS = not studied.
Kruskal–Wallis test, P values: 0·005 (expt 1), 0·0012 (expt 2), <0·0001 (expt 3); *significantly different from mice in control group of the same experiment; **significantly different from mice in group control with syrup of the same experiment; Wilcoxon test, $P<0·05$. Values in the same row with different superscript letters (a, b) are significantly different (Wilcoxon test, $P<0·05$).

were killed by cervical dislocation. The presence of SFBs in the ileum (SFB score) was assessed by light microscopic examination of Gram-stained mucosal scrapings,[11] and was expressed as the percentage of SFB-positive fields; 100 fields were examined at a magnification of × 1000. Caeca with contents were weighed and results (RCW) expressed as percentage of body weight. With Gram-stained microscopic slides (magnification × 1000) of caecal contents the number of fusiforms per 100 bacteria (percentage caecal fusiforms) was determined. On days −1, 1 and 3, the number of faecal Enterobacteriaceae was determined for individual mice.[14]

Statistical analysis

Differences between group means within each experiment were statistically evaluated using the Kruskal–Wallis test. When groups differed significantly, the Wilcoxon test was applied to compare test and control groups. SFB scores within test groups were also compared between experiments. Within each experiment, the number of faecal Enterobacteriaceae for the different days were compared between groups. Kendall correlation coefficients were calculated between SFB scores and the other three parameters. $P<0·05$ was defined as statistically significant.

RESULTS

Antibiotic treatment did not affect body weights (data not shown). Table 1 describes the calculated antibiotic intakes. Daily water consumption per treated mouse ranged between 3 and 10 ml. Water consumption of the control mice given drinking water without additives was 6–8 ml. The control mice with drinking water containing 25 per cent (v:v) syrup drank 10–20 ml/d. Thus, the syrup increased water consumption.

In the treated mice of experiment 1, SFBs were not detectable (Table 2). SFB-harbouring mice were present only in the control group (Table 3). In experiment 2, using 10-fold lower antibiotic concentrations, SFBs were still absent in treated mice except for those treated with either trimethoprim or metronidazole (Table 3). Because of the small numbers of mice per group the inhibitory effect of antibiotics on SFB appearance seen in experiments 1 and 2 could not be substantiated statistically. In experiment 3, all antibiotics, except for gentamicin, trimethoprim and metronidazole, produced significantly reduced SFB scores compared with those of the two control groups ($P<0·0001$). In experiment 3, SFB scores of the two control groups differed significantly ($P=0·004$). SFB scores in mice treated with gentamicin ($P=0·020$) were significantly

Table 3. Influence of antimicrobial drugs on relative caecal weight and percentage fusiform-shaped bacteria in the caecum of mice

Antimicrobial drug†	Relative caecal weight‡			Percentage fusiforms§		
	Expt 1	Expt 2	Expt 3	Expt 1	Expt 2	Expt 3
Amoxycillin	5·4±0·2	2·8±0·0	2·1±0·7	ND	13±11*	33±26*
Doxycyclin	2·7±0·5	2·3±0·0	1·9±0·6	82±23	73±33	60±19
Gentamicin	3·2±0·6	2·5±0·2	2·0±0·5	ND	10±7*	70±12**
Vancomycin	4·9±0·1	3·0±0·0	2·3±0·4	ND	5±0*	52±26
Ciprofloxacin	3·6±1·6	2·6±0·7	1·6±0·5	ND	83±11	81±10**
Trimethoprim	1·5±0·1	1·9±0·4**	2·2±0·6	85±7	75±12*	64±12
Metronidazole	4·5±1·5	2·2±0·4**	1·9±0·3	23±25	64±31	70±12
Clindamycin	3·0±0·7	3·1±0·5	2·0±0·6	25±35	25±35*	83±13*,**
Streptomycin	3·2±0·7	2·8±0·6	NS	21±22	40±22	NS
Cefotaxim	4·5±1·6	2·1±0·4	2·0±0·6	33±46	73±33	75±12**
Control	2·9±0·0	2·1±0·7**	2·1±0·6	95±0	84±12**	68±18
Control with syrup	NS	1·0±0·0	2·0±0·0	NS	50±0	57±9

†See legend to Table 2.
‡Caecum weight expressed as percentage of body weight; means ± SD.
§Number of fusiform-shaped bacteria per 100 bacteria; means ± SD.
ND = not detectable; NS = not studied.
Kruskal–Wallis test, *P* values: relative caecal weight, 0·11 (expt 1), 0·010 (expt 2), 0·20 (expt 3); percentage fusiforms, 0·020 (expt 1), 0·005 (expt 2), 0·0001 (expt 3); *,**see legend to Table 2.

Table 4. Influence of antimicrobial drugs on faecal Enterobacteriaceae in mice

Antimicrobial drug†	Log_{10} (number of Enterobacteriaceae/g faeces)‡								
	Expt 1			Expt 2			Expt 3		
	Day − 1	Day 1	Day 3	Day − 1	Day 1	Day 3	Day − 1	Day 1	Day 3
Amoxycillin	3·0	3·0	1·5	ND	5·5	8·0*	2·5	2·0*	3·3
Doxycyclin	5·0	3·5	2·0	ND	3·0	4·5	4·2	4·0	5·0
Gentamicin	4·0	ND	ND	4·0	3·5	1·5	3·7	5·0	3·0
Vancomycin	4·0	6·5	6·0	3·5	3·0	7·0*	3·5	3·7	3·2
Ciprofloxacin	4·5	ND	ND	2·0	ND	5·0	2·8	ND*	ND*
Trimethoprim	5·0	5·0	2·0	3·8	3·4	3·8	NA	NA	NA
Metronidazole	5·0	3·5	6·0	3·9	4·1	4·3	NA	NA	NA
Clindamycin	4·5	4·5	5·6	3·0	8·0	7·0	3·3	4·3	4·8
Streptomycin	3·2	ND*	ND*	2·0	ND*,**	ND*,**	NS	NS	NS
Cefotaxim	2·5	ND	ND	1·5	4·5	3·5	3·8	2·7*	3·2
Control	4·5	4·5	2·5	4·0	3·6	3·5	5·0	4·7	4·2
Control with syrup	NS	NS	NS	3·0	3·5	4·5	NA	NA	NA

†See legend to Table 2.
‡Group means.
NA = not analysed; ND = not detected; NS = not studied.
Kruskal–Wallis test, *P* values < 0·025; *,**see legend to Table 2.

Table 5. Simplified pattern of sensitivity to antimicrobial drugs of SFBs, compared to that of facultatively anaerobic and obligately anaerobic bacteria

Antimicrobial drug	Sensitivity of: Facultative anaerobes* Gram +ve	Facultative anaerobes* Gram −ve	Obligate anaerobes* Gram +ve	Obligate anaerobes* Gram −ve	Degree of sensitivity of SFBs†
Amoxycillin	+	v	+	−	+++
Doxycyclin	+	+	+	−	+++
Gentamicin	v	+	−	−	++
Vancomycin	+	−	+	−	++
Ciprofloxacin	+	+	−	−	+++
Trimethoprim	+	v	−	−	+
Metronidazole	−	−	+	+	+
Clindamycin	v	v	+	+	+++
Streptomycin	+	v	−	−	++‡
Cefotaxim	+	+	+	−	+++

*+, Sensitive; −, insensitive; v, variation in sensitivity between bacterial species (source: McEvoy, GK (ed). AHFS Drug Information 90, American Society of Hospital Pharmacists, 1990).
†+, Elimination of SFBs by dose ≥therapeutic dose (TD); ++, elimination by dose $\geq TD \times 10^{-1}$; +++, elimination by dose $\geq TD \times 10^{-2}$.
‡Sensitivity of SFBs to streptomycin given at a dose $TD \times 10^{-2}$ not tested.

higher in experiment 3 than in experiments 1 and 2. In experiments 2 and 3, mice given either trimethoprim or metronidazole, had significantly higher SFB scores than in experiment 1.

Irrespective of the dose used, amoxycillin, doxycyclin, ciprofloxacin, clindamycin, streptomycin and cefotaxim induced absence of SFBs in mucosal smears of all treated mice (Table 2). The addition of syrup to the drinking water did not influence the incidence of SFB-positive mice.

Table 3 shows that RCW was not systematically affected by the antibiotics. Doxycyclin and trimethoprim did not alter percentage caecal fusiforms ($P < 0{\cdot}05$). Lowering the dosage of the other antibiotics was associated with increased percentage fusiforms. At the lowest dosage (expt 3), the antibiotics did not decrease percentage caecal fusiforms, except for amoxicillin.

Table 4 illustrates that antibiotic treatment differently influenced the number of faecal Enterobacteriaceae. At the lowest dosage (expt 3) only ciprofloxacin systematically reduced this parameter, whereas at the highest dosage gentamicin, streptomycin and cefotaxim also did so.

DISCUSSION

This study clearly shows that SFB scores, RCW, caecal fusiforms and faecal Enterobacteriaceae respond differently to antibiotic administration. This is supported by the fact that SFB scores and none of the three parameters were significantly correlated. Differential responsiveness to external factors for SFB scores and either RCW or caecal fusiforms was previously reported.[12,13] As to a correlation between SFBs and Enterobacteriaceae, there are conflicting studies.[12,13,21] Thus, the antibiotics studied may have direct effects on SFB scores either by inhibiting SFB colonisation or by killing SFBs.

SFBs were sensitive to amoxycillin, doxycyclin, vancomycin, ciprofloxacin, clindamycin and cefotaxim, even given in lower doses than used normally by other investigators.[8,29] Streptomycin given at therapeutic concentrations[7,8,18,25,26,29] or 10 times lower also eliminated SFBs. The high sensitivity of SFBs to some of the antibiotics tested agrees with earlier findings.[4,18,20,28] It has been reported that roxythromycin, erythromycin, neomycin, streptomycin, bacitracin, the combination trimethoprim-sulphamethoxazole, and polymyxin induce disappearance of SFBs from the ileum of mice.[18,20] Within 10·5 h of access to drinking water containing 0·6 mg/ml penicillin, mice were free from SFBs.[4] SFBs were scarcely present in the ileum of broiler chickens treated with virginiamycin.[28]

Table 5 summarises the observed *in vivo* sensitivity of SFBs to antibiotics, compared to the *in vitro*

sensitivity of facultative and obligate anaerobes. SFBs were inhibited by doses of ciprofloxacin as low as 1 per cent of the therapeutic dose, whereas obligately anaerobic bacteria are insensitive.[2,3] This also holds for gentamicin[2,23] and streptomycin,[2,23] whereas these drugs, given at 10 per cent of the therapeutic mouse dose, inhibited SFBs. Thus, SFBs behave unlike obligate anaerobes.

Based on the sensitivity pattern and the fact that the habitat of SFBs is the relatively anaerobic ileum,[3] they can be considered facultatively anaerobic with high sensitivity to antibiotics.

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